STUDIES IN THE FERTILIZATION OF PHYCOMYCETES.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XLII.

FRANK LINCOLN STEVENS.

(WITH PLATE XVII)

SCLEROSPORA, a genus of the Peronosporales, stands in a way intermediate between the Peronosporaceae and the Albuginaceae, a position that renders the problem of gametogenesis and fertilization of particular interest. This genus has heretofore received no cytological investigation.

The oogonia of Sclerospora average about 45 to 50μ in diameter and are irregularly spherical in shape. The oogonial wall, even in young stages, is much thickened, thus forming a marked contrast with either Peronospora or Albugo. This wall averages from 3 to 5μ in thickness, and stains readily with orange G.

Each oogonium contains approximately forty to fifty nuclei, borne in a cytoplasm differing in no essential particular from that of the oogonium of Albugo (*fig.* 1). The nuclei are relatively large and are rather fewer in number in comparison with the size of the oogonium than is usual with the other Peronosporales. The earliest stages of oogonium formation were not observed, so that it is not known whether the peculiar streaming of cytoplasm and the distortion of nuclei observed in Albugo (Istvánffi '95, Wager '96, Stevens '99) and in Peronospora (Wager '00) obtains here. As in Albugo, the nuclei in the young oogonium rapidly enlarge and pass into the spirem condition (*fig.* 1). From this stage on the process resembles that in *A. candida* (Wager '96, Davis '00, Stevens '01) more closely than in other Peronosporales, yet with certain marked differences. The mitosis is closely simultaneous, and may proceed until near metaphase without any indication of approaching zonation. When meta-

phase is reached, however, the nuclei are found arranged in an approximate circle around the region that is to become the oosphere (*fig. 2*). The arrangement is that of *A. candida* rather than of *A. Bliti*, and is such that no daughter nuclei can re-enter the oosphere region.

The exodus of nuclei is not complete, otherwise the oosphere would be enucleate and the condition would be like that of *A. Bliti*. One nucleus and, so far as my observation goes, one only remains behind with the coenocentrum, agreeing in this particular with *A. candida*. This nucleus may be seen there later in mitosis (*fig. 2*). This stage is directly comparable with that of Albugo represented by *fig. 14* in Stevens '01, with the difference that zonation is delayed longer in Sclerospora than in *A. candida*. The sequel of this condition is given in *fig. 3*, were one supernumerary nucleus is wandering toward the periplasm, leaving the female pronucleus in contact with or imbedded in the coenocentrum.

During the completion of the mitosis the ooplasm and periplasm become clearly differentiated, but as yet no plasmoderma exists. This process of differentiation has been termed zonation, and is here accomplished by a withdrawal of cytoplasm from the periphery of the oogonium, as in Peronospora (Wager '00, *fig. 2*) and *Albugo candida* (Stevens '01, *fig. 14*), not by an aggregation of separate dense regions, as in *A. Bliti* (Stevens '99, *figs. 59–61*).

At all times during oogenesis there is maintained in the oogonial wall contiguous to the antheridium an unthickened region (*figs. 1, 4, 5*).

In all the early stages of oogenesis the oosphere is seen to be eccentric, indeed the plasmoderma is usually almost in contact with that side of the oogonium adjacent to the antheridium (*fig. 4*). In cases of shrinkage it is also observed that the oogonial contents adhere firmly to this side of the oogonium, a phenomenon to be observed also in Albugo.

The coenocentrum is shown in *figs. 2–4*, and a trace of it still persists in *fig. 5*. In Sclerospora this structure is not so definite as in *Albugo candida* and *A. Tragopogonis*. Its highest differentiation is shown in *fig. 2*, where it consists merely of a region of

dense cytoplasm in the center of the oogonium, differentiated into two portions of varying density. In history and structure the coenocentrum agrees well with that of *A. Bliti*, with the exception that the central globule has not yet been demonstrated.

In function the coenocentrum serves to attract and to retain the female pronucleus, as in *A. Tragopogonis* and *A. candida* (Stevens '01), and in *Peronospora parasitica* (Wager '00). No evidence of nutritive function was apparent as in the case of the forms of Albugo just mentioned.

The mitosis in Sclerospora is in general of the type described for *A. Bliti* (Stevens '99). The spindle is intranuclear, the membrane persisting until metasphase. No centrosomes or polar radiations were seen. Chromosomes were not counted with accuracy, but they are few in number, probably four.

The antheridium in Sclerospora is usually very small, and is closely appressed to the oogonial wall (*fig. 4*). Usually it is impossible to distinguish the antheridial wall from the oogonial, and very frequently the thinning of the oogonial wall gives the antheridium the appearance of being imbedded in it. The antheridial nuclei enlarge simultaneously with those of the oogonium, and undergo mitosis. No receptive papilla was seen, and this peculiar structure may be lacking in the genus.

Communication is opened between the antheridium and the oogonium by the bulging and eventual rupturing of the oogonial wall at the thin point between these organs. The edges of the ruptured wall thus extend inward. The antheridial tube, which is soft and gelatinous, penetrates the oosphere, discharging its contents before it reaches the center (*fig. 4*). The track of the antheridial tube remains in evidence for some time as a denser region in the cytoplasm.

The male pronucleus is slightly smaller than the female and is more ovoid in form (*fig. 7*). It migrates to the female and fuses with it, but the fusion was not studied critically owing to the smallness of the nuclei.

The oogonial wall, as stated above, is much thickened even in the youngest oogonia, and it does not change in dimensions

as it matures. With zonation the regions of ooplasm and periplasm are clearly mapped (*fig. 3*), and with the opening of the antheridial tube the plasmoderma appears. The oospore wall now develops rapidly, first appearing as a clearly defined wall (*fig. 5*), then rapidly increasing in thickness (*fig. 6*). As the oospore wall thickens the oogonial wall collapses in irregular folds (*fig. 6*), and the periplasm and periplasmic nuclei degenerate. The protection afforded by these two thick walls appears to hinder seriously the penetration of killing and fixing fluids or subsequent manipulation, and no good preparations showing nuclei were obtained at any stage after the second wall was far developed.

GENERAL CONSIDERATIONS.

The general bearing of the cytological evidence on the relationship of Sclerospora is to emphasize its affinity to the Peronosporaceae rather than to the Albuginaceae, and to indicate its specialized rather than primitive character. The general course of development of both antheridium and oogonium, and the behavior of the periplasm are as in the whole group Peronosporales. The mitosis in both of these organs has been shown by Trow ('99, '01), Hartog ('95), Wager ('96, '00), Miyake ('01), Stevens ('99, '01), King ('02), and others, to be characteristic of both Saprolegniales and Pernosporales.

The mode of zonation in Sclerospora is not that of the Saprolegniales (Trow '01, Miyake '01, Hartog '95), nor of the primitive Albuginaceae (Stevens '99). It does agree closely, however, with the Peronosporaceae. While the zonation of *A. candida* agrees with that of the Peronosporaceae, Sclerospora shows by the nature of its coenocentrum, which in structure, history, and function is clearly like that found in Peronospora, its closer relationship to the Peronosporaceae than to any Albugo.

The conclusion regarding the relationship of the genus, purely from cytological standpoint, coincides with that drawn from the gross study of asexual spore-bearing organs, and emphasizes the relationship of this genus to Peronospora, of which genus it was once considered a member.

Sclerospora, as shown by the combined evidence of the

double investment of its oospore, the character of its conidiophores, and its cytology, probably separated early from the main line of the Peronosporaceae, at a time much later than the divergence of the Albuginaceae from the Peronosporaceae.

It is a pleasure to acknowledge indebtedness to my wife for the plate accompanying this article.

COLLEGE OF AGRICULTURE AND MECHANIC ARTS.
Raleigh, N. C.

LITERATURE CITED.

DAVIS, '00: The fertilization of *Albugo candida*. BOT. GAZ. **29**: 297–311.

HARTOG, '95: On the cytology of the vegetative and reproductive organs of Saprolegniaceae. Trans. Roy. Irish Acad. **30**: 649.

ISTVÁNFFI, '95: Ueber die Rolle der Zellkerns bei der Entwickelung der Pilze. Ber. Deut. Bot. Gesell. **13**: 456.

KING, '02: Fertilization and some accompanying phenomena in *Araiospora pulchra*, one of the aquatic Phycomycetes. Science **15**: 456.

MIYAKE, '01: The fertilization of *Pythium de Baryanum*. Ann. Botany **15**: 653–667.

STEVENS, '99: The compound of oosphere of *Albugo Bliti*. BOT. GAZ. **28**: 149–176.

——— '01: Gametogenesis and fertilization in Albugo. BOT. GAZ. **32**: 77–98.

TROW, '99: Observation on the biology and cytology of a new variety of *Achlya americana*. Ann. Botany **13**: 131–179.

——— '01: Observations on the biology and cytology of *Pythium ultimum*, n. sp. Ann. Botany **15**: 269–312.

WAGER, '96: On the structure and reproduction of *Cystopus candidus* Lev. Ann. Botany **10**: 295–342.

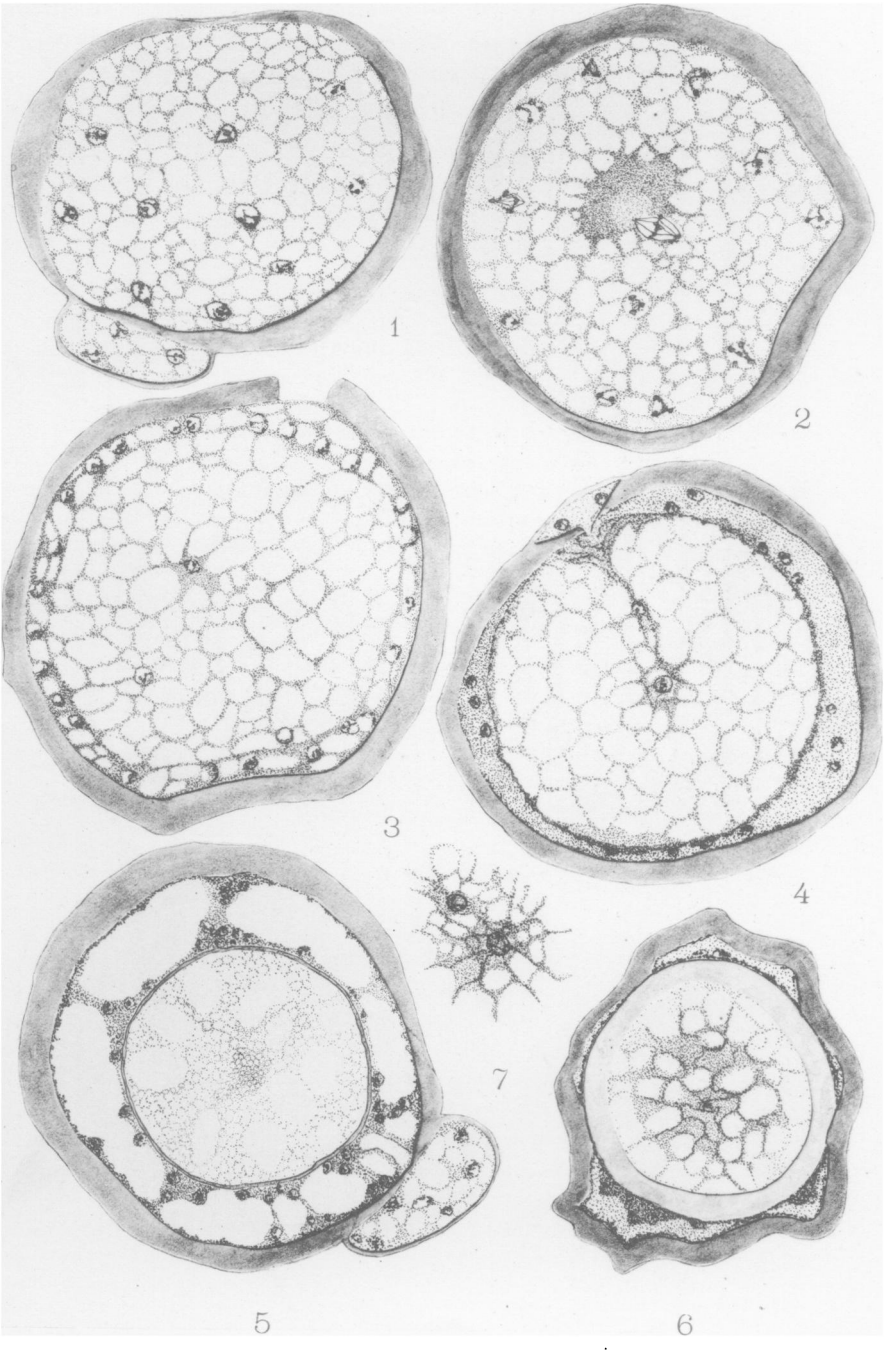
——— '00: On the fertilization of *Peronospora parasitica*. Ann. Botany **14**: 263–273.

EXPLANATION OF PLATE XVII.

All figures are from material killed in chrome-acetic acid and stained with Flemming's triple stain. The figures were sketched with the aid of an Abbé camera, using the Leitz $\frac{1}{12}$ objective, ap. 1.30. The original drawings gave an enlargement of 1790 diameters, and have been reduced one-third in reproduction.

FIG. 1. Young oogonium and antheridium; nuclei in spirem; wall thickened and staining darkly with orange G.

FIG. 2. Nuclei migrating to periphery; coenocentrum well developed; one nucleus in metaphase anchored to it; peripheral nuclei in advanced prophase.



STEVENS on SCLEROSPORA.

FIG. 3. Zonation; mitosis completed; one daughter nucleus remaining with coenocentrum, the other passing to periplasm.

FIG. 4. Fertilization; antheridial tube ruptured, emptying one nucleus into oosphere; female nucleus resting in coenocentrum.

FIG. 5. Oospore wall forming; periplasm and periplasmic nuclei degenerating; remains of antheridium and trace of the tube in periplasm.

FIG. 6. Winter condition.

FIG. 7. Pronuclei just before fusion.